

SEQUENCE LISTING

1528960
DT01 Rec'd PCT/PTO 22 MAR 2005

<110> DSM IP ASSETS B.V.

<120> Enone reductase gene

<130> NDR5231

<140> PCT/EP03/10473

<141> 2003-09-19

<150> EP 02021098.5

<151> 2002-09-23

<160> 32

<170> PatentIn version 3.2

<210> 1

<211> 1212

<212> DNA

<213> Candida kefyr

<220>

<221> CDS

<222> (1)..(1212)

<400> 1

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Met	Ser	Tyr	Met	Asn	Phe	Asp	Pro	Lys	Pro	Leu	Gly	Asp	Thr	Asn	Ile	
1				5					10					15		

ttc	aag	cca	atc	aag	atc	ggt	aac	aat	gag	cta	aaa	cac	aga	gta	gtc	96
Phe	Lys	Pro	Ile	Lys	Ile	Gly	Asn	Asn	Glu	Leu	Lys	His	Arg	Val	Val	
			20					25					30			

atg	cca	gca	ttg	act	aga	atg	aga	gcc	att	gca	cca	gga	aac	atc	cca	144
Met	Pro	Ala	Leu	Thr	Arg	Met	Arg	Ala	Ile	Ala	Pro	Gly	Asn	Ile	Pro	
		35					40					45				

aac	act	gaa	tgg	gcc	gag	gaa	tac	tac	aga	caa	cgt	tct	caa	tac	cct	192
Asn	Thr	Glu	Trp	Ala	Glu	Glu	Tyr	Tyr	Arg	Gln	Arg	Ser	Gln	Tyr	Pro	
	50				55						60					

ggt	acc	ctt	att	atc	acg	gaa	ggt	act	ttc	cct	tct	gcg	caa	tca	ggt	240
Gly	Thr	Leu	Ile	Ile	Thr	Glu	Gly	Thr	Phe	Pro	Ser	Ala	Gln	Ser	Gly	
65					70					75					80	

ggt	tac	cca	aat	gtg	cca	ggt	atc	tgg	tcc	aaa	gag	caa	ttg	gct	gaa	288
Gly	Tyr	Pro	Asn	Val	Pro	Gly	Ile	Trp	Ser	Lys	Glu	Gln	Leu	Ala	Glu	
				85				90						95		

tgg	aaa	aag	atc	ttc	aat	gca	atc	cat	gag	aac	aaa	tcg	ttc	gtg	tgg	336
Trp	Lys	Lys	Ile	Phe	Asn	Ala	Ile	His	Glu	Asn	Lys	Ser	Phe	Val	Trp	
			100					105					110			

gtg	caa	ttg	tgg	gtt	cta	ggt	aga	caa	gca	tgg	cca	gaa	gtg	ttg	aag	384
Val	Gln	Leu	Trp	Val	Leu	Gly	Arg	Gln	Ala	Trp	Pro	Glu	Val	Leu	Lys	
		115					120					125				

aag	gaa	ggt	ttg	cgt	tac	gat	agt	gct	acc	gat	gac	ttg	tac	atg	ggt	432
Lys	Glu	Gly	Leu	Arg	Tyr	Asp	Ser	Ala	Thr	Asp	Asp	Leu	Tyr	Met	Gly	
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Lys Lys Tyr

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<212> PRT
<213> Candida kefyr

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Phe Lys Pro Ile Lys Ile Gly Asn Asn Glu Leu Lys His Arg Val Val
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Met Pro Ala Leu Thr Arg Met Arg Ala Ile Ala Pro Gly Asn Ile Pro
35 40 45

Asn Thr Glu Trp Ala Glu Glu Tyr Tyr Arg Gln Arg Ser Gln Tyr Pro
50 55 60

Gly Thr Leu Ile Ile Thr Glu Gly Thr Phe Pro Ser Ala Gln Ser Gly
65 70 75 80

Gly Tyr Pro Asn Val Pro Gly Ile Trp Ser Lys Glu Gln Leu Ala Glu
85 90 95

Trp Lys Lys Ile Phe Asn Ala Ile His Glu Asn Lys Ser Phe Val Trp
100 105 110

Val Gln Leu Trp Val Leu Gly Arg Gln Ala Trp Pro Glu Val Leu Lys
115 120 125

Lys Glu Gly Leu Arg Tyr Asp Ser Ala Thr Asp Asp Leu Tyr Met Gly
130 135 140

Glu Glu Glu Lys Glu Arg Ala Leu Lys Ala Asn Asn Pro Gln His Gly
145 150 155 160

Ile Thr Lys Glu Glu Ile Lys Gln Tyr Ile Lys Glu Tyr Val Asp Ala
165 170 175

Ala Lys Lys Ala Ile Asp Ala Gly Ala Asp Gly Val Gln Ile His Ser
180 185 190

Ala Asn Gly Tyr Leu Leu Asn Gln Phe Leu Asp Pro Ile Ser Asn Asn
195 200 205

Arg Thr Asp Glu Tyr Gly Gly Ser Ile Glu Asn Arg Ala Arg Phe Thr
210 215 220

Leu Glu Val Val Asp Ala Val Val Asp Ala Val Gly Ala Glu Arg Thr
225 230 235 240

Ser Ile Arg Phe Ser Pro Tyr Gly Thr Phe Gly Thr Met Ser Gly Gly
245 250 255

Glu Asn Pro Gly Ile Val Ala Gln Tyr Ala Tyr Val Ile Gly Glu Leu
260 265 270

Glu Lys Arg Ala Arg Ala Gly Lys Arg Leu Ala Phe Ile Asp Leu Val
275 280 285

Glu Pro Arg Val Thr Asp Pro Phe Leu Pro Glu Phe Glu Lys Trp Phe
290 295 300

Lys Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp Lys Gly Pro Val
305 310 315 320

Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln Ala Thr Leu Asp
325 330 335

Ser Lys Lys Pro Asn Thr Leu Ile Gly Tyr Gly Arg Ser Phe Ile Ala
340 345 350

Asn Pro Asp Leu Val Tyr Arg Leu Glu Lys Gly Leu Pro Leu Asn Lys
355 360 365

Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys Glu Gly Tyr Thr Asp
370 375 380

Tyr Pro Ser Tyr Glu Glu Ser Val Ala Lys Gly Tyr Lys Lys Glu Glu
385 390 395 400

Lys Lys Tyr

<210> 3
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<212> PRT
<213> Candida kefyr

<400> 3

Pro Leu Gly Asp Thr Asn Ile Phe Lys Pro Ile Lys
1 5 10

<210> 4
<211> 50
<212> PRT
<213> Candida kefyr

<400> 4

His Arg Val Val Met Pro Ala Leu Thr Arg Met Arg Ala Ile Ala Pro
1 5 10 15

Gly Asn Ile Pro Asn Thr Glu Trp Ala Glu Glu Tyr Tyr Arg Gln Arg
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Ser Gln Tyr Pro Gly Thr Leu Ile Ile Thr Glu Gly Thr Phe Pro Ser
35 40 45

Val Gln
50

<210> 5

<211> 8

<212> PRT

<213> Candida kefyr

<400> 5

Glu Gln Leu Ala Glu Trp Lys Lys
1 5

<210> 6

<211> 9

<212> PRT

<213> Candida kefyr

<400> 6

Ile Phe Asn Ala Ile His Glu Asn Lys
1 5

<210> 7

<211> 18

<212> PRT

<213> Candida kefyr

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<221> MISC_FEATURE

<222> (15)..(15)

<220>

<221> MISC_FEATURE

<222> (15)..(15)

<223> Xaa is unknown

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Ser Phe Val Trp Val Gln Leu Trp Val Leu Gly Arg Gln Ala Xaa Pro
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Glu val

<210> 8
<211> 19
<212> PRT
<213> Candida kefyr

<400> 8

Glu Gly Leu Arg Tyr Asp Ser Ala Phe Asp Asp Leu Tyr Met Gly Glu
1 5 10 15

Glu Glu Lys

<210> 9
<211> 10
<212> PRT
<213> Candida kefyr

<400> 9

Ala Asn Asn Pro Gln His Gly Ile Thr Lys
1 5 10

<210> 10
<211> 7
<212> PRT
<213> Candida kefyr

<400> 10

Glu Tyr Val Asp Ala Ala Lys
1 5

<210> 11
<211> 50
<212> PRT
<213> Candida kefyr

<220>
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<222> (43)..(43)
<223> Xaa is unknown

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<223> Xaa is unknown

<220>
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<222> (47)..(48)
<223> Xaa is unknown

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Ala Ile Asp Ala Gly Ala Asp Gly Val Gln Ile His Ser Ala Asn Gly
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Tyr Leu Leu Asn Gln Phe Leu Asp Pro Ile Ser Asn Asn Arg Thr Asp
20 25 30

Glu Tyr Gly Gly Ser Ile Ile Asn Arg Ala Xaa Phe Xaa Leu Xaa Xaa
35 40 45

Val Asp
50

<210> 12
<211> 22
<212> PRT
<213> Candida kefyr

<400> 12

Arg Leu Ala Phe Ile Asp Leu Val Glu Pro Arg Val Thr Asp Pro Phe
1 5 10 15

Leu Pro Glu Phe Glu Lys
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<210> 13
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<213> Candida kefyr

<400> 13

Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp Lys
1 5 10

<210> 14
<211> 21
<212> PRT
<213> Candida kefyr

<400> 14

Gly Pro Val Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln Ala
1 5 10 15

Thr Leu Asp Ser Lys
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<210> 15
<211> 24
<212> PRT
<213> Candida kefyr

<400> 15

Leu Pro Asn Thr Leu Ile Gly Tyr Gly Arg Ser Phe Ile Ala Asn Pro
1 5 10 15

Asp Leu Val Tyr Arg Leu Glu Lys

<210> 16
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 <212> PRT
 <213> Candida kefyr

<400> 16

Gly Leu Pro Leu Asn Lys
 1 5

<210> 17
 <211> 11
 <212> PRT
 <213> Candida kefyr

<400> 17

Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys
 1 5 10

<210> 18
 <211> 15
 <212> PRT
 <213> Candida kefyr

<400> 18

Glu Gly Tyr Thr Asp Tyr Pro Ser Tyr Glu Glu Ser Val Ala Lys
 1 5 10 15

<210> 19
 <211> 9
 <212> PRT
 <213> Candida kefyr

<400> 19

Gly Asp Thr Asn Ile Phe Lys Pro Ile
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<210> 20
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 <212> PRT
 <213> Artificial Sequence

<220>
 <223> reverse sequence of a partial amino acid sequence of enone reductase

<400> 20

Gly Glu Lys Thr Phe Thr Tyr Phe Thr
 1 5

<210> 21
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 <212> DNA

<213> Candida kefyr

<220>

<221> CDS

<222> (1)..(1056)

<400> 21

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His	Arg	Val	Val	Met	Pro	Ala	Leu	Thr	Arg	Met	Arg	Ala	Ile	Ala	Pro	
1				5					10					15		

gga	aac	atc	cca	aac	act	gaa	tgg	gcc	gag	gaa	tac	tac	aga	caa	cgt	96
Gly	Asn	Ile	Pro	Asn	Thr	Glu	Trp	Ala	Glu	Glu	Tyr	Tyr	Arg	Gln	Arg	
			20					25					30			

tct	caa	tac	cct	ggg	acc	ctt	att	atc	acg	gaa	ggg	act	ttc	cct	tct	144
Ser	Gln	Tyr	Pro	Gly	Thr	Leu	Ile	Ile	Thr	Glu	Gly	Thr	Phe	Pro	Ser	
		35					40					45				

gcg	caa	tca	ggg	ggg	tac	cca	aat	gtg	cca	ggg	atc	tgg	tcc	aaa	gag	192
Ala	Gln	Ser	Gly	Gly	Tyr	Pro	Asn	Val	Pro	Gly	Ile	Trp	Ser	Lys	Glu	
	50					55					60					

caa	ttg	gct	gaa	tgg	aaa	aag	atc	ttc	aat	gca	atc	cat	gag	aac	aaa	240
Gln	Leu	Ala	Glu	Trp	Lys	Lys	Ile	Phe	Asn	Ala	Ile	His	Glu	Asn	Lys	
65					70					75					80	

tcg	ttc	gtg	tgg	gtg	caa	ttg	tgg	gtt	cta	ggg	aga	caa	gca	tgg	cca	288
Ser	Phe	Val	Trp	Val	Gln	Leu	Trp	Val	Leu	Gly	Arg	Gln	Ala	Trp	Pro	
				85					90					95		

gaa	gtg	ttg	aag	aag	gaa	ggg	ttg	cgt	tac	gat	agt	gct	acc	gat	gac	336
Glu	Val	Leu	Lys	Lys	Glu	Gly	Leu	Arg	Tyr	Asp	Ser	Ala	Thr	Asp	Asp	
			100					105					110			

ttg	tac	atg	ggg	gaa	gaa	gaa	aaa	gag	cgt	gcc	tta	aag	gct	aac	aac	384
Leu	Tyr	Met	Gly	Glu	Glu	Glu	Lys	Glu	Arg	Ala	Leu	Lys	Ala	Asn	Asn	
		115					120					125				

cca	cag	cac	ggg	atc	acc	aag	gaa	gaa	atc	aag	cag	tac	atc	aag	gag	432
Pro	Gln	His	Gly	Ile	Thr	Lys	Glu	Glu	Ile	Lys	Gln	Tyr	Ile	Lys	Glu	
	130					135					140					

tac	gtg	gat	gct	gcc	aag	aaa	gcc	atc	gat	gca	ggg	gca	gac	ggg	gtg	480
Tyr	Val	Asp	Ala	Ala	Lys	Lys	Ala	Ile	Asp	Ala	Gly	Ala	Asp	Gly	Val	
145					150					155					160	

caa	atc	cat	tct	gcc	aac	ggg	tac	ttg	ttg	aac	cag	ttt	ttg	gac	cct	528
Gln	Ile	His	Ser	Ala	Asn	Gly	Tyr	Leu	Leu	Asn	Gln	Phe	Leu	Asp	Pro	
				165					170					175		

att	tct	aac	aac	aga	acc	gac	gag	tac	ggg	gga	tcg	atc	gag	aac	cgt	576
Ile	Ser	Asn	Asn	Arg	Thr	Asp	Glu	Tyr	Gly	Gly	Ser	Ile	Glu	Asn	Arg	
			180					185					190			

gcg	aga	ttc	act	ttg	gaa	gtg	gtt	gat	gcc	gtt	gta	gat	gca	gtt	ggg	624
Ala	Arg	Phe	Thr	Leu	Glu	Val	Val	Asp	Ala	Val	Val	Asp	Ala	Val	Gly	
		195					200					205				

gcc	gaa	aga	acc	tcc	atc	aga	ttc	tct	cct	tac	ggg	act	ttt	ggg	acc	672
Ala	Glu	Arg	Thr	Ser	Ile	Arg	Phe	Ser	Pro	Tyr	Gly	Thr	Phe	Gly	Thr	
	210					215					220					

atg	tcc	ggg	ggg	gag	aac	cct	ggc	atc	gtt	gcc	caa	tat	gca	tac	gtc	720
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Met 225	Ser	Gly	Gly	Glu	Asn 230	Pro	Gly	Ile	Val	Ala 235	Gln	Tyr	Ala	Tyr	Val 240	
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atc Ile	gat Asp	ttg Leu	gtc Val 260	gag Glu	cct Pro	cgt Arg	gtg Val 265	acc Thr	gac Asp	cca Pro	ttc Phe	cta Leu	cca Pro 270	gaa Glu	ttc Phe	816
gag Glu	aag Lys	tgg Trp 275	ttc Phe	aag Lys	gaa Glu	ggt Gly	acc Thr 280	aac Asn	gaa Glu	ttc Phe	atc Ile	tac Tyr 285	tct Ser	atc Ile	tgg Trp	864
aag Lys	ggt Gly 290	cca Pro	gtt Val	ctc Leu	aga Arg	gtt Val 295	ggt Gly	aac Asn	tat Tyr	gct Ala 300	ttg Leu	gac Asp	cca Pro	gat Asp	caa Gln	912
gct Ala 305	act Thr	atc Ile	gac Asp	tct Ser	aag Lys 310	aag Lys	cct Pro	aac Asn	acc Thr	ttg Leu 315	atc Ile	ggt Gly	tac Tyr	ggt Gly	aga Arg 320	960
tcc Ser	ttt Phe	att Ile	gcc Ala	aac Asn 325	cca Pro	gac Asp	ttg Leu	gtg Val	tac Tyr 330	cgt Arg	ttg Leu	gaa Glu	aag Lys	ggt Gly 335	ttg Leu	1008
cca Pro	ttg Leu	aac Asn 340	aag Lys	tat Tyr	gat Asp	aga Arg	aac Asn	acc Thr 345	ttc Phe	tac Tyr	acc Thr	ttc Phe	acc Thr 350	aaa Lys	gag Glu	1056
gg																1058

<210> 22
 <211> 352
 <212> PRT
 <213> Candida kefyr

<400> 22

His 1	Arg	Val	Val	Met 5	Pro	Ala	Leu	Thr	Arg 10	Met	Arg	Ala	Ile	Ala 15	Pro
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Ser	Gln	Tyr 35	Pro	Gly	Thr	Leu	Ile 40	Ile	Thr	Glu	Gly	Thr 45	Phe	Pro	Ser
Ala 50	Gln	Ser	Gly	Gly	Tyr	Pro 55	Asn	Val	Pro	Gly	Ile 60	Trp	Ser	Lys	Glu
Gln 65	Leu	Ala	Glu	Trp	Lys 70	Lys	Ile	Phe	Asn 75	Ala	Ile	His	Glu	Asn 80	Lys
Ser	Phe	Val	Trp	Val 85	Gln	Leu	Trp	Val	Leu 90	Gly	Arg	Gln	Ala	Trp 95	Pro

Glu Val Leu Lys Lys Glu Gly Leu Arg Tyr Asp Ser Ala Thr Asp Asp
 100 105 110
 Leu Tyr Met Gly Glu Glu Glu Lys Glu Arg Ala Leu Lys Ala Asn Asn
 115 120 125
 Pro Gln His Gly Ile Thr Lys Glu Glu Ile Lys Gln Tyr Ile Lys Glu
 130 135 140
 Tyr Val Asp Ala Ala Lys Lys Ala Ile Asp Ala Gly Ala Asp Gly Val
 145 150 155 160
 Gln Ile His Ser Ala Asn Gly Tyr Leu Leu Asn Gln Phe Leu Asp Pro
 165 170 175
 Ile Ser Asn Asn Arg Thr Asp Glu Tyr Gly Gly Ser Ile Glu Asn Arg
 180 185 190
 Ala Arg Phe Thr Leu Glu Val Val Asp Ala Val Val Asp Ala Val Gly
 195 200 205
 Ala Glu Arg Thr Ser Ile Arg Phe Ser Pro Tyr Gly Thr Phe Gly Thr
 210 215 220
 Met Ser Gly Gly Glu Asn Pro Gly Ile Val Ala Gln Tyr Ala Tyr Val
 225 230 235 240
 Ile Gly Glu Leu Glu Lys Arg Ala Arg Ala Gly Lys Arg Leu Ala Phe
 245 250 255
 Ile Asp Leu Val Glu Pro Arg Val Thr Asp Pro Phe Leu Pro Glu Phe
 260 265 270
 Glu Lys Trp Phe Lys Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp
 275 280 285
 Lys Gly Pro Val Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln
 290 295 300
 Ala Thr Ile Asp Ser Lys Lys Pro Asn Thr Leu Ile Gly Tyr Gly Arg
 305 310 315 320
 Ser Phe Ile Ala Asn Pro Asp Leu Val Tyr Arg Leu Glu Lys Gly Leu
 325 330 335
 Pro Leu Asn Lys Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys Glu
 340 345 350

<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> IA1 (antisense primer for upstream region)

<400> 23
attcctcggc ccattcagtg ttggg 25

<210> 24
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> IS1 (sense primer for downstream region)

<400> 24
ggtgtaccgt ttggaaaagg gtttgc 26

<210> 25
<211> 1796
<212> DNA
<213> Candida kefyr

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aattaattat atcaacaaac tgtcgagatg tcgtacatga actttgaccc taagccattg 180
ggagacacca atatcttcaa gccaatcaag atcggtaaca atgagctaaa acacagagta 240
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ggtactttcc cttctgcgca atcagggtgtt tacccaaatg tgccaggtat ctggtccaaa 420
gagcaattgg ctgaatggaa aaagatcttc aatgcaatcc atgagaacaa atcgttcgtg 480
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gccaaaccag acttggtgta ccgtttggaa aagggtttgc cattgaacaa gtatgataga	1260
aacaccttct acaccttcac caaagaaggt tacaccgatt acccaagcta cgaggaatcc	1320
gtcgcaaagg gttacaagaa agaggaaaag aagtactaag cttgaactga taactagcca	1380
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tggcagatga ggggcctttg accttcaatg tttgtatgta agtaatgatt caaaagattc	1560
tcctctatag ttggttagtt actactaaaa gaaaccttca atataaaaca atgatcgaga	1620
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ctaccattaa gtctttgtgc gaaatgagta accttatatt aataaaaagat gtgaagtgtg	1740
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<210> 26
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense Primer

<400> 26	
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<210> 27
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense Primer

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<210> 28
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 <212> DNA
 <213> Candida kefyr

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ggtaacaatg agctaaaaca cagagtagtc atgccagcat tgactagaat gagagccatt	180
gcaccaggaa acatcccaaa cactgaatgg gccgaggaat actacagaca acgttctcaa	240
taccctggta cccttattat cacggaaggt actttccctt ctgcgcaatc aggtgggttac	300

ccaaatgtgc caggtatctg gtccaaagag caattggctg aatggaaaaa gatcttcaat	360
gcaatccatg agaacaaatc gttcgtgtgg gtgcaattgt gggttctagg tagacaagca	420
tggccagaag tgttgaagaa ggaagggttg cgttacgata gtgctaccga tgacttgtac	480
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gcagggtgcag acggtgtgca aatccattct gccaacgggt acttggtgaa ccagtttttg	660
gaccctatth ctaacaacag aaccgacgag tacgggtggat cgatcgagaa ccgtgcgaga	720
ttcacttttg aagtggtcga tgccgttgtc gatgcagttg gtgccgaaag aacctccatc	780
agattctctc catacggtag ttttggtagc atgtccggtg gtgagaacct tggcatcggt	840
gctcaatatg catacgtcat tggtagagtg gaaaagagag ctagagctgg caagagattg	900
gcgttcacgc atttggtcga gcctcgtgtg accgacccat tcctaccaga attcgagaag	960
tgggtcaagg aaggtaccaa cgaattcatc tactctatct ggaagggtcc agttctcaga	1020
gttggttaact atgctttgga cccagatcaa gccactctcg actctaagaa gcctaacact	1080
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